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(71) Applicant *(for all designated States except US)*: PROTEUS HOUSE [GB/GB]; Lyme Green Business Park, Macclesfield, Cheshire SK11 0JL (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : FISHLEIGH, Robert, Vincent [GB/GB]; Bradley Smithy Cottage, Gurnett, Macclesfield, Cheshire SK11 0HD (GB). GREANEY, Paul, John [GB/GB]; Flat No. 6, 6 Lancaster Road, Didsbury, Manchester M20 8TY (GB). ROBSON, Barry [GB/GB]; The Old Bakery, 22A Town Street, Marple Bridge, Marple, Cheshire SK6 5AA (GB). SHAKESPEARE, Ann, Patricia [GB/GB]; 4 St. George's Avenue, Timperley, Altrincham, Cheshire WA15 6HE (GB).

(74) Agent: FRANK B. DEHN & CO.; Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).

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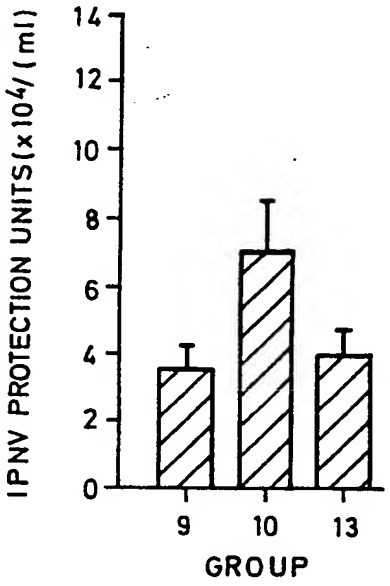
(57) Abstract

The invention provides a synthetic polypeptide having at least one antigenic property of at least one strain of infectious pancreatic necrosis virus, said polypeptide consisting substantially of an amino acid sequence selected from formulae (I) to (IX): formula (I) (which contains Seq. ID No: 1) X-Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Y; formula (II) (which contains Seq. ID Nos: 2 or 3) X-Thr-Asp-Phe-R_1-Ser-Asp-Leu-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Y; formula (III) (which contains Seq. ID No: 4) X-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Trp-Arg-Asp-Y;fFormula (IV) (which contains Seq. ID No: 5) X-Thr-Lys-Tyr-Gly-Lys-Tyr-Asp-Pro-Glu-Gly-Y; formula (V) (which contains Seq. ID No: 6) X-Leu-Glu-Val-Ser-Glu-Ser-Gly-Ser-Gly-Y; formula (VI) (which contains Seq. ID Nos: 7 or 8) X-Gln-Glu-Thr-Ser-Ser-Tyr-R_2-Leu-Glu-Val-Ser-Glu-Ser-Gly-Y; formula (VII) (which contains Seq. ID Nos: 9 or 10) X-Ser-Arg-Phe-Thr-Pro-Ser-Gly-Asp-R_3-Y; and formula (VIII) (which contains Seq. ID Nos: 11 or 12) X-Pro-His-Gln-Glu-Pro-Ala-Pro-R_4-Phe-Tyr-Y; formula (IX) (which contains Seq. ID Nos: 13, 14, 15 or 16) X-Pro-Gln-Gly-R_5-Gln-Ser-Met-Asn-Gly-Ala-R_6-Y, wherein R_1 is Ser or Thr; R_2 is Thr or Asn; R_3 is Asp-Gly or Asn-Ala; R_4 is Asp-Asp or Glu-Glu-; R_5 is Pro or Leu; R_6 is Arg or Lys; and X and Y may each independently be absent or independently be one or more amino acid residues, useful for the treatment of IPNV infection in fish.



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IPNV VACCINE

This invention relates to synthetic polypeptides, more particularly polypeptides which emulate the antigenic properties of specific regions of proteins of infectious pancreatic necrosis virus (IPNV).

IPNV is the aetiological agent of infectious pancreatic necrosis, an acute, contagious, high mortality disease in young, hatchery-reared salmonids. The virus was first isolated from Brook trout (Salvelinus fontinalis) and has subsequently been found to cause disease in a number of economically important salmonid and non-salmonid species, especially fish under the age of six months. Those species considered most susceptible include rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), cut-throat trout (Salmo clarki), amago trout (Oncorhynchus rhodurus) sockeye salmon (Oncorhynchus nerka), Arctic char (Salvelinus alpinus) Atlantic Salmon (Salmo salar) and the Japanese eel (Anguilla japonica). The disease has also been found in striped bass and the virus is also harboured by many types of non-salmonid species of which only a few have proved to be susceptible to the disease, e.g. pike.

The name of the disease refers to the degeneration and severe necrosis of exocrine pancreas revealed by histopathological examination although fish suffering from the disease show the following clinical signs: loss of equilibrium with whirling and corkscrew swimming followed by death, and a characteristically swollen belly.

Since IPNV survivors can become carriers while producing circulating antibodies, they can shed virus in faeces and thereby lead to lateral transmission of the virus. Vertical transmission also occurs resulting from adsorption of the virus to egg shells or to spermatozoa

with penetration of the egg at fertilization.

A recent estimate for the total output of farmed North Atlantic Salmon was approximately 225,000 tonnes per annum with the main producer being Norway farming around 160,000 tonnes each year at a value of around $15 million. Other major producing countries of salmonids are France, UK, USA, Canada, Australia, Spain, Germany, New Zealand and Japan. It has been calculated that the world market in salmon is set to grow by an average of 7.5% every year until the year 2000 and result in a turnover worth $3,600 million per annum. However, outbreaks of IPNV have been reported in Canada, Denmark, Finland, France, Germany, Greece, Japan, Netherlands, South Africa, Spain, Sweden, Switzerland, UK and USA. Although IPNV is a disease predominantly found in fish, the virus has also been isolated from mollusks (Hill, B.J. (1976). Wildlife diseases, ed. L.A. Page, 445-452, Plenum Press, New York) and crustaceans. Thus, the disease is widespread and is a threat to profitable fish farming.

Attempts have been made to vaccinate fish against IPNV using inactivated or attenuated virus but several of the vaccines based on inactivated virus have been unsuccessful. Moreover, vaccines based on attenuated virus do not appear to confer protection and carry the possibility of reverting to virulent types of virus. An avirulent strain, (74/53) isolated from perch in England, has given reproducible results but protection fluctuated between 25 and 75% depending on route of administration.

IPNV is a member of the family Birnaviradae, a group which consists of medium-sized, unenveloped icosahedral animal viruses with bisegmented double stranded RNA genomes. The larger segment, segment A, encodes a polyprotein containing VP2 and VP3, VP2 being the major capsid protein and VP3 being an internal protein.

An object of the invention is the development of synthetic polypeptides which can elicit the production of antibodies, preferably neutralizing antibodies, to the VP2 and VP3 proteins of IPNV.

Our invention provides, in a first aspect, a synthetic polypeptide having at least one antigenic property of at least one strain of infectious pancreatic necrosis virus, said polypeptide consisting substantially of an amino acid sequence selected from sequences of Formulae (I) to (IX):

Formula (I) (which contains Seq. ID No: 1)

X-Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Y;

Formula (II) (which contains Seq. ID Nos: 2 or 3)

X-Thr-Asp-Phe-R_1-Ser-Asp-Leu-Pro-
Thr-Ser-Lys-Ala-Trp-Gly-Y;

Formula (III) (which contains Seq. ID No: 4)

X-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Trp-Arg-Asp-Y;

Formula (IV) (which contains Seq. ID No: 5)

X-Thr-Lys-Tyr-Gly-Lys-Tyr-Asp-Pro-Glu-Gly-Y;

Formula (V) (which contains Seq. ID No: 6)

X-Leu-Glu-Val-Ser-Glu-Ser-Gly-Ser-Gly-Y;

Formula (VI) (which contains Seq. ID Nos: 7 or 8)

X-Gln-Glu-Thr-Ser-Ser-Tyr-R_2-Leu
-Glu-Val-Ser-Glu-Ser-Gly-Y;

Formula (VII) (which contains Seq. ID Nos: 9 or 10)

X-Ser-Arg-Phe-Thr-Pro-Ser-Gly-Asp-R_3-Y; and

Formula (VIII) (which contains Seq. ID Nos: 11 or 12)

X-Pro-His-Gln-Glu-Pro-Ala-Pro-R_4-Phe-Tyr-Y

Formula (IX) (which contains Seq. ID Nos: 13, 14, 15 or 16)

X-Pro-Gln-Gly-R_5-Gln-Ser-Met-Asn-Gly-Ala-R_6-Y

wherein

R_1 is Ser or Thr;

R_2 is Thr or Asn;

R_3 is Asp-Gly or Asn-Ala;

R_4 is Asp-Asp or Glu-Glu-;

R_5 is Pro or Leu;

R_6 is Arg or Lys; and

X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not form an epitope or part of an epitope of the VP2 or VP3 proteins of any strain of IPNV which, in the sequence of the VP2 or VP3 proteins of the strain, is contiguous with the sequence to which X and Y are attached; the polypeptide optionally containing a functionalised coupling moiety.

It is preferred that R_1 is Ser, R_2 is Thr and R_3 is Asp-Gly; R_4 may be either Asp-Asp or Glu-Glu. Also it is preferred that R_6 is Arg when R_5 is Pro and that R_6 is Lys when R_5 is Leu.

The sequences to which X and Y are attached (hereinafter "the core sequences") in the polypeptide sequences of Formulae (I) to (IX) above were chosen on the basis of their topographical similarity to one or more antigenic determinants of VP2 and VP3 proteins of IPNV. In particular, Formulae (I) to (VI) and (IX) relate to VP2 and Formulae (VII) and (VIII) relate to VP3.

Peptides according to Formulae (I) to (IX) above without X and Y being present are useful, for example, in the production of antibodies to VP2 and VP3. When X or Y are present they may be any length but preferably less than 20 amino acids each, more preferably less than 10, eg. 1 to 6.

Particularly preferably, if X or Y are present they are relatively short sequences, typically 1 to 3

residues long. In most instances, either Y is absent and X is 1 or 2 residues long, or X is absent and Y is one or two residues long.

The peptides in accordance with the invention are especially effective when coupled to a carrier. The polypeptides of the invention may be coupled to a carrier by any conventional method. The point of attachment of the polypeptide of the invention to the carrier may be in the core sequence, but is preferably in the X or Y sequences.

Amino acids which have side chains suitable for direct coupling to a carrier molecule are, for example, Cys, Lys and Tyr. Alternatively, coupling may be achieved, for example, by means of a functionalised coupling moiety. Such a moiety may be introduced into the polypeptides of the invention, for example, by chemically modifying (i.e. functionalising) an amino acid so that a functional group is introduced specifically for the purpose of facilitating coupling to a carrier. Examples of functional groups useful in coupling to a carrier include thiol, amino, hydrazino or hydrazide, and aldehyde or masked aldehyde groups.

A functionalised coupling moiety may also be introduced into the polypeptides of the invention, for example, by reaction with a heterobifunctional coupling reagent such as N-γ-maleimidobutyryloxy-succinimide which can be used, e.g. to couple a lysine residue in the carrier to a C-terminal or other cysteine residue in a polypeptide (Kitagawa, T. & Ackawa, T. (1976) J. Biochem. 79, 233). Other coupling reactions and reagents which, for example, either become incorporated into the final conjugate or activate certain reactive sites of the carrier protein molecule for subsequent linkage with the peptide have been described in the literature. Where heterobifunctional reagents are employed in coupling reactions, the site of attachment of the carrier to the polypeptide of the invention will

generally be in the X or Y sequences so as to take advantage of the high specificity of these reagents.

Whichever method of coupling is employed, it is preferred that the site of attachment is in the X or Y sequences and also that the antigenic sections of the polypeptides are spaced from the carrier in order to optimise their effect. Spacing may be achieved, for example, by including additional amino acids (such as Gly) in X or Y between the coupling site and the antigenic section, or alternatively the desired spacing can be obtained by employing a suitable heterobifunctional coupling reagent.

Preferably, where X or Y consists of a single amino acid residue, that amino acid provides a suitable site for coupling to a carrier. Preferably, where X or Y consists of two amino acids, one amino acid provides a suitable site for coupling to a carrier and the other residue (e.g. Gly) acts as a spacer. In Formulae (I), (III), (VIII) and (IX), it is particularly preferred that X is absent and Y is Gly-Cys and in Formulae (II), and (IV) to (VII), it is particularly preferred that X is absent and Y is Cys.

Suitable carriers include, for example, tetanus toxoid, cholera toxin and its B subunit, ovalbumin, chicken gamma globulin (CGG), soybean trypsin inhibitor, muramyl dipeptide and analogues thereof, and Braun's lipoprotein although other suitable carriers will be readily apparent to the skilled person. For example, multiple antigen peptides (MAPs) may be used such as those comprising a polylysyl core, e.g. heptalysyl, bearing reactive amino termini. Polypeptide antigens according to the invention may be reacted with, or synthesised on, the amino termini and different polypeptide antigens may be reacted with the same core or carrier.

It is also envisaged that existing fish vaccines may be used as carriers or adjuvants for synthetic

polypeptide antigens according to the invention. The following microorganisms or macromolecular subunits therefrom may serve as suitable carriers or adjuvants: Aeromonas salmonicida, Yersinia ruckeri, vibrio anguillarum, Vibrio ordalii and Renibacterium salmoniarum.

The microorganism may be, for example, present as whole killed cells, inactivated cells (e.g. formalin treated) or live attenuated cells. Macromolecular subunits include, for example, disrupted cells (bacterins), extra cellular products (ECP) and toxoids thereof, and purified antigens from natural or synthetic sources. Vaccines against furunculosis, the disease caused by the bacterium Aeromonas salmonicida, which show promise include whole killed or disrupted cells, ECP and ECP toxoids and whole killed cells in combination with ECP, live attenuated cells and purified antigens. In addition, hyperimmune sera to certain antigens raised in fish or mammals have been used for passive protection. A successful vaccine against enteric redmouth (ERM) caused by the bacterium Yersinia ruckeri has been produced which comprises formalin-inactivated whole bacteria. Current commercial vibriosis vaccines in the Northern Hemisphere contain mixtures of the most common species, Vibrio anguillarum and Vibrio ordalii. These vaccines are simple inactivated cultures containing mixtures of whole cells and ECP.

It will of course be appreciated that the sequences according to Formulae (I) to (IX) may constitute a protein with X and Y being major portions of the protein with the antigenic sequence being, for example, part of an exposed loop on a globular protein.

Whilst the above polypeptides in accordance with the invention are preferred as they are especially effective in eliciting the production of highly specific antibodies, it is nevertheless envisaged that

polypeptides in which X and Y do form an epitope which, in the VP2 or VP3 protein of an IPNV strain is contiguous with the sequence to which X and Y are attached would also be effective. Thus in such polypeptides, the region which is homologous with and/or topographically similar to natural IPNV sequence will extend beyond the core sequence into the adjacent residues in the X and Y sequences so as to include the adjacent epitope or epitopes. However, if the region of homology with the natural sequence is too long, the polypeptides will be less effective e.g. because antibodies produced in response to them may be of lower specificity. In a separate aspect therefore, the invention provides a polypeptide of Formulae (I) to (IX) as defined above wherein R_1, R_2, R_3, R_4, R_5 and R_6 are as defined above and X and Y may each independently be absent or each independently represent one or more amino acids with the proviso that, if X or Y are such that the region of homology with the sequence of the VP2 or VP3 protein of any strain of IPNV is extended into the X or Y sequences, the region of homology must not be so long as to have a significantly detrimental effect on the efficacy of the polypeptide as a vaccine and/or the ability of the polypeptide to elicit antibodies of high specificity. Preferably the region of homology does not extend beyond the core sequence by more than, for example, 20 amino acids in either the X sequence or the Y sequence. In particularly preferred polypeptides, the region of homology does not extend by more than 10 (e.g. 6) amino acids into either the X or Y sequences.

Peptides according to the invention may be synthesised by any suitable method, for example by use of either standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry (see, for example, Atherton, E. and Sheppard, R. C. (1985) J. Chem. Soc. Chem. Comm., 165) or standard t-butyloxycarbonate (t-Boc) chemistry. The correctness of the structure and the level of purity, which will

normally be in excess of 95%, should be carefully checked. Various chromatographic analyses, including reverse phase high performance liquid chromatography, and spectrographic analyses, including mass spectrometry may for example be employed for this purpose. In order to facilitate high yields and good purity, peptides made using Fmoc chemistry may be N-terminally acetylated and/or C-terminally amidated, and such modifications are included within the scope of the present invention (Stuber, W., Knolle, J. & Breipohl, G. (1989). Synthesis of peptide amides by Fmoc-solid-phase peptide synthesis on acid labile anchor groups. Int. J. Pept. & Prot. Res., 34, 215-221).

All the sequences herein are stated using the standard I.U.P.A.C. three-letter-code abbreviations for amino acid residues defined as follows: Gly-Glycine, Ala-Alanine, Val-Valine, Leu-Leucine, Ile-Isoleucine, Ser-Serine, Thr-Threonine, Asp-Aspartic acid, Glu-Glutamic acid, Asn-Asparagine, Gln-Glutamine, Lys-Lysine, His-Histidine, Arg-Arginine, Phe-Phenylalanine, Tyr-Tyrosine, Trp-Tryptophan, Cys-Cysteine, Met-Methionine and Pro-Proline.

Polypeptides according to the invention may be used to raise antibodies which will cross-react with VP2 and VP3 proteins produced by a range of IPNV strains. Our analyses have shown that, since the conformational/topographic/electrostatic properties of polypeptides according to the invention are such that they are highly likely to elicit the production of antibodies which will cross-react with VP2 and VP3 proteins from several or many strains, further advantages may arise from combining several variant polypeptides in a larger polypeptide. Such a polypeptide may have the general Formula (A):

$$[L_a\text{-}F]_m\text{-}[L_b\text{-}G]_n\text{-}L_c \qquad (A)$$

wherein F and G may each independently be a polypeptide according to any one of Formulae (I) to (IX) L is a linking sequence, a, b and c are each independently 0 or 1 and m and n are each positive numbers e.g. between 1 and 10 inclusive. L is preferably a short, conformationally flexible section of polypeptide chain such as, for example and without limit Gly-Gly-Gly-Gly-Gly (Seq. ID No: 17), Gly-Pro-Gly-Pro-Gly-Pro (Seq. ID No: 18) or Gly-Ser-Ala-Gly-Ser-Gly-Ala (Seq. ID No: 19). It should be clear that each repeat may optionally have a different variant of a polypeptide according to the invention.

It should be noted that there is homology between the C-terminal portion of Formula (II) and the N-terminal portion of Formula (III) and between the C-terminal portion of Formula (VI) and the N-terminal portion of Formula (V). Advantage may be taken of this homology when producing larger polypeptides according to Formula (A). Linking sequences together with respective X and Y moieties may be omitted and the residues in brackets may be selected so that either regions of homology are duplicated or some or all of the duplicated residues are omitted. In the latter case it will be seen that the C-terminal portion of one polypeptide merges with the N-terminal portion of the other polypeptide.

Polyvalent determinant analogues as defined by Formula (A) are referred to as pseudohomopolyvalent, wherein variants of essentially the same determinant analogue are repeated in a single polypeptide chain. In addition, simple homopolyvalent polypeptide immunogens, which contain multiple copies of the same variant of one of the determinant analogues according to any one of Formula (I) to (IX), are also effective, and are also included within the scope of the present invention.

Pseudohomopolyvalent immunogenic polypeptides are particularly valuable as vaccines, where they elicit the

production of a range of (neutralising) antibodies with a similar but non-identical underlying specificity, which between them cross-react with VP2 or VP3 proteins from a wider range of IPNV strains, and are thus more effective at conferring protective immunity. There are also advantages in constructing heteropolyvalent polypeptides which contain one or more copies, in any order, of one of the polypeptides according to the present invention and one or more other polypeptide analogues or determinant analogues. Such polypeptides, which are provided for in the present invention, have the general Formula (B):

$$L_d\text{-}[G\text{-}L]_m\text{-}F\text{-}[L\text{-}G]_n\text{-}L_e \qquad (B)$$

wherein F is a polypeptide according to any one of Formulae (I) to (IX), G is a polypeptide according to any one of Formulae (I) to (IX) or other sequence, m and n are each positive numbers e.g. between 1 and 10 inclusive, and d and e are each independently 0 or 1. "L" is preferably a short, conformationally flexible section of polypeptide chain such as, for example and without limit Gly-Gly-Gly-Gly-Gly (Seq. ID No: 17), Gly-Pro-Gly-Pro-Gly-Pro (Seq. ID No: 18) or Gly-Ser-Ala-Gly-Ser-Gly-Ala (Seq ID No: 19).

It is to be understood that any antigenically significant subfragments and/or antigenically significant variants of the above-identified polypeptide sequences which retain the general form and function of the parent polypeptide are included within the scope of this invention. The substitution of any of the specific residues by residues having comparable conformational and/or physical properties, including substitution by rare amino acids (e.g. D-stereoisomers) or synthetic amino acid analogues, is included within the scope of the invention. For example, substitution of a residue by another in the same Set, as defined below, is

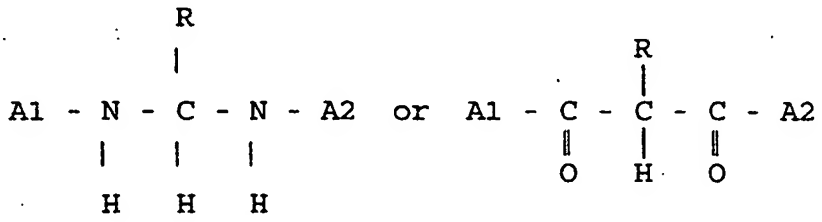
included within the ambit of the invention; Set 1 - Ala, Val, Leu, Ile, Phe, Tyr, Trp and Met; Set 2 - Ser, Thr, Asn and Gln; Set 3 - Asp and Glu; Set 4 - Lys, His and Arg; Set 5 - Asn and Asp; Set 6 - Glu and Gln; Set 7 - Gly, Ala, Pro, Ser and Thr. D-stereoisomers of all amino acid types, may be substituted, for example, D-Phe, D-Tyr and D-Trp.

Embodiments of the invention, in which X and/or Y are present may independently include one or more segments of protein sequence with the ability to act as a T-cell epitope. For example, segments of amino acid sequence of the general formula 1-2-3-4, where 1 is Gly or a charged amino acid (e.g. Lys, His, Arg, Asp or Glu), 2 is a hydrophobic amino acid (e.g. Ile, Leu, Val, Met, Tyr, Phe, Trp, Ala), 3 is either a hydrophobic amino acid (as defined above) or an uncharged polar amino acid (e.g. Asn, Ser, Thr, Pro, Gln, Gly), and 4 is a polar amino acid (e.g. Lys, Arg, His, Glu, Asp, Asn, Gln, Ser, Thr, Pro), appear to act as T-cell epitopes in at least some instances (Rothbard, J.B. & Taylor, W.R. (1988); "A sequence pattern is common to T-cell epitopes." The EMBO Journal 7(1): 93-100).

Similarly segments can be of the sequence 1'-2'-3'-4'-5', wherein 1' is equivalent to 1 as defined earlier, 2' to 2, 3' and 4' to 3, and 5' to 4 (ibid). Both forms are included within the scope of the present invention and one or more T-cell epitopes (preferably less than five) may be incorporated into a polypeptide according to the invention. The or each epitope may be of the type defined above or may be of other structure and may be separated by spacer segments of any length or composition (preferably less than five amino acid residues in length) and comprise for example residues selected from Gly, Ala, Pro, Asn, Thr, Ser or polyfunctional linkers such as non-α amino acids. It is possible for a C- or N-terminal linker to represent a complete protein, thus obviating the possible need for

conjugation to a carrier protein.

Also included within the scope of this invention are analogues of the polypeptide according to Formulae (I) to (IX) in which X or Y are or include a "retro-inverso" amino acid, i.e. a bifunctional amine or bifunctional carboxyl. For example an analogue according to the invention and containing a retro-inverso amino acid may have the formula:



where R is any amino acid side chain, e.g. a glycine side chain, or other functional group, and A1 and A2 are preferably each at least one synthetic polypeptide according to the invention or other peptide sequences, e.g. having desirable antigenic properties (but not necessarily the same) attached by its N- or C-terminal end. T-cell epitopes may optionally be included in A1 or A2 as discussed earlier.

Retro-inverso modification of peptides involves the reversal of one or more peptide bonds to create analogues more resistant than the original molecule to enzymatic degradation and offer one convenient route to the generation of branched immunogens which contain a high concentration of epitope for a medium to large immunogen. The use of these compounds in large-scale solution synthesis of retro-inverso analogues of short-chain biologically active peptide is of particular interest.

It should be noted that analogues incorporating retro-inverso amino acid derivatives cannot be made directly using a recombinant DNA system. However, the basic analogues can be synthesised by recombinant means

and they can then be purified and chemically linked to the retro-inverso amino-acids using standard techniques of peptide/organic chemistry. A practical and convenient novel procedure for the solid-phase synthesis on polyamide-type resin of retro-inverso peptides has been described recently [Gazerro, H., Pinori, M. & Verdini, A.S. (1990); "A new general procedure for the solid-phase synthesis of retro-inverso peptides"; a section of "Innovation and Perspectives in Solid Phase Synthesis" Ed. Roger Epton, SPCC (UK) Ltd, Birmingham, UK].

After the young fish have hatched, the specific immune system is not fully mature for several weeks since the major lymphoid organs in teleost fish, the thymus, kidney and spleen, are not fully functioning. The thymus is the first lymphoid organ to develop lymphocytes and gives rise to 'T' lymphocytes. 'B' lymphocytes are also present, but the exact source of this cell type in teleost fish is not known. It would be desirable to challenge the fish immune system with antigen as soon as it is sufficiently mature to give rise to immunity.

Several methods exist for the administration of fish vaccines: e.g. by injection, the oral route and via immersion.

Intraperitoneal injection is an effective method of vaccination and furthermore permits the use of adjuvants to enhance the magnitude of the immune response. Disadvantages are that fish require anaesthetization and handling which cause stress, and the process is also very labour intensive. However, by using repeater syringes and a production line system, 1000 fish can be injected per hour. However, intraperitoneal injection cannot be used on fish much below 15 g. Vaccines against furunculosis, ERM and vibriosis can all be administered by intraperitoneal injection.

Oral vaccination is suitable for mass

administration to fish of all sizes and imposes no stress due to handling. However, intrinsic limitations exist whereby relatively large amounts of vaccines are required, increasing cost and uncertainty of individual dosage. It is preferred to provide oral vaccines in the form of a food composition or food supplement which is e.g. granular or in pellet form. The specific gravity of the oral vaccine will be dependent upon the feeding habits of the fish and the nature of their containment, although it may be desirable for example, to form the granules or pellets with air voids so that they float initially but sink when the voids fill with water.

Direct Immersion (D.I.) is simple and rapid, requiring only a few seconds of exposure to the vaccine. This method is now automated and a 'bath' or 'flush' variation was developed for vibriosis and ERM vaccines, and simply involves pouring the vaccine into holding tanks. Although this method consumes more vaccine and requires longer exposure (about one hour), which involves oxygenation of the water and close monitoring of fish for stress, it is less labour intensive than injection.

Intraperitoneal injection, oral vaccination and direct immersion are all well suited to administering vaccine compositions comprising polypeptides according to the present invention.

It may be advantageous to immunise with a cocktail containing (i) a given polypeptide conjugated to more than one type of carrier, and/or (ii) more than one kind of polypeptide conjugated to the same carrier. Moreover, various polypeptides, their conjugates, and cocktails thereof may be administered in any suitable adjuvant or delivery system, and more than one adjuvant or delivery system may be combined to form a so-called "super-cocktail". Preferred adjuvants and delivery systems include microspheres, liposomes, micelles, niosomes, ISCOMS.

In order to elicit antibody formation in non-fish subjects, the polypeptides, either alone or linked to a carrier molecule, may be administered by any route (eg parenteral, nasal, oral, rectal, intra-vaginal), with or without the use of conventional adjuvants (such as aluminium hydroxide or, in the case of laboratory animals, Freund's complete or incomplete adjuvants) and/or other immunopotentiating agents. In our co-pending PCT Application No. PCT/GB93/00716, filed on 7 April 1993, we disclose non-ionic surfactant vesicles (i.e. niosomes) which may be used advantageously as adjuvants with peptides according to the present invention. The invention also includes formulation of polypeptides according to the invention in slow-release forms, such as a sub-dermal implant or depot comprising, for example, liposomes (Allison, A.C. & Gregoriadis, G. (1974) Nature (London) 252, 252) or biodegradable microcapsules manufactured from co-polymers of lactic acid and glycolic acids (Gresser, J. D. and Sanderson, J. E. (1984) in "Biopolymer Controlled Release Systems" pp 127-138, Ed. D. L. Wise).

Again, in some circumstances it will be advantageous to immunise with a cocktail containing (i) a given polypeptide conjugated to more than one type of carrier molecule, and/or (ii) more than one kind of polypeptide conjugated to the same carrier molecule. Moreover, any of the polypeptides, their conjugates, and cocktails thereof may be administered in any suitable adjuvant or delivery system, and more than one adjuvant or delivery system may be combined to form a so-called "super-cocktail".

It is to be understood that the polypeptides according to the invention may be synthesised by any conventional method, either directly using manual or automated peptide synthesis techniques as mentioned above, or indirectly by RNA or DNA synthesis and conventional techniques of molecular biology and genetic

engineering. Such techniques may be used to produce hybrid proteins containing one or more of the polypeptides inserted into another polypeptide sequence.

Another aspect of the present invention therefore provides a DNA molecule coding for at least one synthetic polypeptide according to the invention, preferably incorporated into a suitable expression vector replicable in microorganisms or in mammalian, insect, plant, fungal or other cells. The DNA may also be part of the DNA sequence for a longer product e.g. the polypeptides may be expressed as parts of other proteins into which they have been inserted by genetic engineering. One practical guide to such techniques is "Molecular cloning: a laboratory manual" by Sambrook, J., Fritsch, E.F. and Maniatis, T. (2nd Edition, 1989).

Polypeptides according to the invention may be used either alone or linked to an appropriate carrier, as:

(a) Peptide vaccines, for use to prevent infection by one or more strains of IPNV;

(b) As ligands in assays of, for example, serum from IPNV positive subjects;

(c) As antigens for <u>in vitro</u> cellular bioassays e.g. interferon and lymphokine assays which detect interferon or lymphokines released in response to specific antigen in a whole blood culture or the lymphocyte proliferation assay;

(d) As quality control agents in testing, for example, binding levels of antibodies raised against the polypeptides; and

(e) As immunogenic agents for the generation of monoclonal or polyclonal antibodies by immunisation of an appropriate animal, such antibodies being of use for (i) the scientific study of the IPNV, and (ii) as diagnostic agents, e.g. as part of histochemical reagents. The invention further provides for genetically engineered forms or sub-components, especially V_H regions, of antibodies raised against the

polypeptides, and of piscinised forms of antibodies initially raised against the polypeptides in other animals, using techniques described in the literature.

In respect of detection and diagnosis of IPNV or antibodies against IPNV, the skilled person will be aware of a variety of immunoassay techniques known in the art, inter alia, sandwich assay, competitive and non-competitive assays and the use of direct and indirect labelling.

A further apsect of the invention provides a method of detecting IPNV or antibodies against IPNV or antigen binding fragments thereof, which comprises incubating a sample of tissue or body fluid of a fish with at least one polypeptide according to the invention and determining whether, and if desired the extent to which and/or rate at which cross-reaction between said sample and said polypeptide occurs.

A further aspect of the invention provides a kit for detecting IPNV or antibodies against IPNV which comprises at least one synthetic polypeptide according to the invention. In some instances it will be desirable to include a mixture of polypeptides according to the invention in the kit. Such kits may also comprise support means (e.g. plastic, polystyrene, latex or red blood cells) and/or a means of detecting binding of antibodies or antigen binding fragments to the synthetic polypeptides (e.g. fluorescent-, radio- or enzyme-labelled anti-IgG antibodies).

The preparation of polyclonal or monoclonal antibodies, recombinant forms particularly adapted to the species of interest, e.g. piscinised forms of antibodies (see, for example, Thompson K. M. et al (1986) Immunology 58, 157-160), single domain antibodies (see, for example, Ward, E. S., Gussow, D., Griffiths, A. D., Jones, P. and Winter, G. (1989) Nature 341, 544-546), which bind specifically to a synthetic polypeptide according to the present invention, may be

carried out by conventional means and such antibodies are considered to form part of this invention.

Antibodies according to the invention are, inter alia, of use in a method of diagnosing IPNV infection in fish which comprises incubating a sample of tissue or body fluid of said fish with an effective amount of antibody or antigen binding fragment thereof as described herein and determining whether, and if desired the extent to which and/or rate at which, cross-reaction between said sample and said antibody occurs. Diagnostic kits which contain at least one of said antibodies also form part of this invention. Such kits may comprise one or more of the following: support means (e.g. as discussed above); antibodies or antigen binding fragments thereof according to the invention; at least one synthetic polypeptide according to the invention; and a means for detecting binding of antibodies or antigen binding fragments to said synthetic polypeptide.

The use of synthetic polypeptides or antibodies or antigen binding fragments thereof according to the invention for the detection of IPNV or antibodies against IPNV on histological sections also form part of this invention.

Antibodies raised by immunisation using a synthetic polypeptide according to the invention can be used to raise anti-idiotypic antibodies which also form part of this invention. In another aspect, there is provided a process for the preparation of an anti-idiotypic antibody which comprises immunising a mammal with an antibody or antigen binding fragment thereof which binds specifically to a synthetic polypeptide according to the invention, and isolating the anti-idiotypic antibody formed or cells which produce anti-idiotypic antibody.

A further aspect of the invention provides synthetic polypeptides as defined above for use in stimulating the piscine immune system for therapy or prophylaxis of IPNV infection in fish and for the

preparation of medicaments suitable for such uses. Also included are pharmaceutical, especially vaccine, compositions containing, as active ingredient, at least one polypeptide or polypeptide-carrier conjugate as described herein in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients. Additionally are included pharmaceutical, especially vaccine, compositions containing, as active ingredient, an antibody or antigen binding fragment thereof which binds specifically to a synthetic polypeptide or polypeptide coupled to a carrier according to the present invention, in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients. The compositions may be formulated for oral, direct immersion or especially parenteral administration.

The invention further provides a method of stimulating the piscine immune system for therapy or prophylaxis of IPNV infection in fish, which comprises administering an effective amount of a polypeptide as hereinbefore defined to a piscine subject.

Also provided is a method for the therapy or prophylaxis of IPNV infection in fish which comprises administering an effective amount of an antibody or antigen binding fragment thereof which binds specifically to a synthetic polypeptide or polypeptide coupled to a carrier according to the present invention.

The following non-limiting Examples illustrate the invention.

Description of the Drawings

Figure 1 illustrates antibody titres obtained at 8 weeks post immunisation of fish in accordance with the procedure described below;

Figure 2 illustrates antibody titres obtained at 20 weeks post immunisation; and

Figures 3 and 4 illustrate serum neutralisation 8 weeks post immunisation.

EXAMPLE A

A peptide having the sequence:

Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Gly-Cys

Formula (I) in which X is absent and Y is -Gly-Cys; Seq. I.D. No: 20 is synthesised using the standard solid phase Fmoc methodologies. The peptide is cleaved from the resin in the presence of trifluoroacetic acid and subsequent purification is achieved by gel filtration, ion exchange chromatography and reverse phase high performance liquid chromatography. The purity of the resultant peptide is in excess of 85%. The peptide is conjugated to a carrier, chicken gamma globulin (CGG) by MBS (m-Maleimide benzoyl-N-hydroxysuccinimide ester) and mixed with Freund's Complete Adjuvant (FCA).

Rabbits are inoculated subcutaneously with this preparation and challenged with a further similar quantity in Freund's Incomplete Adjuvant (FIA) 4 and 8 weeks later. Blood samples are taken 2 weeks after the third injection and antisera assayed for neutralizing activity.

Strains of West Buxton, Sp, Ab and Canada 1 serotypes of IPNV (described by Caswell-Reno, P. et al (1986), Journal of General Virology, 67: 2193-2205) are propagated in Chinook salmon embryo cells (CHSE-214;

ATCC No. CRL 1681) at 20°C in Eagle's MEM supplemented with 10% foetal bovine serum. The virus is then concentrated by polyethylene glycol precipitation, extracted with freon 113 and purified by isopycnic CsCl-gradient centrifugation.

Neutralization assay

Test antisera is inactivated by heat at 56°C for 30 minutes and doubling dilutions are prepared. Aliquots of the dilutions are mixed with an equal volume of growth medium containing 2 x 10^3 $TCID_{50}$/ml virus and incubated at room temperature for thirty minutes. 0.1 ml of each virus/antiserum mixture is then added to each of four wells of a 96-well microtitration plate with monolayer cultures of CHSE-214 cells in 0.1 ml growth medium. Cytopathic effects are determined after incubation at 20°C for 7 days and the 50% neutralizing dose is calculated.

EXAMPLE B

Peptide Synthesis.

The following C-terminal extended IPNV peptides were synthesised using standard Fmoc solid phase chemistry [Atherton, E. & sheppard, R.C. (1985). J. Chem. Soc. Commun. 165-166]. All peptides were N-terminally acetylated.

1a Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Gly-Cys (Formula (I) in which X is absent and Y is Gly-Cys; Seq. ID No. 20)

2a Thr-Asp-Phe-Ser-Ser-Asp-Leu-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Cys (Formula (II) in which R_1 is Ser, X is absent and Y is Cys; Seq. ID No. 21)

3a Pro-Thr-Ser-Lys-Ala-Trp-Gly-Trp-Arg-Asp-Gly-Cys (Formula (III) in which X is absent and Y is Gly-Cys; Seq. ID No. 22)

4a Thr-Lys-Tyr-Gly-Lys-Tyr-Asp-Pro-Glu-Gly-Cys (Formula (IV) in which X is absent and Y is Cys; Seq. ID No. 23)

5a Leu-Glu-Val-Ser-Glu-Ser-Gly-Ser-Gly-Cys (Formula (V) in which X is absent and Y is Cys; Seq. ID No. 24)

6a Gln-Glu-Thr-Ser-Ser-Tyr-Thr-Leu-Glu-Val-Ser-Glu-Ser-Gly-Cys (Formula (VI) in which R_2 is Thr, X is absent and Y is Cys; Seq. ID No. 25)

7a Ser-Arg-Phe-Thr-Pro-Ser-Gly-Asp-Asp-Gly-Cys (Formula (VII) in which R_3 is Asp-Gly, X is absent and Y is Cys; Seq. ID No. 26)

8a Pro-His-Gln-Glu-Pro-Ala-Pro-Asp-Asp-Phe-Tyr-Gly-Cys (Formula (VIII) in which R_4 is Asp-Asp, X is absent and Y is Gly-Cys; Seq. ID No. 27)

8b Pro-His-Gln-Glu-Pro-Ala-Pro-Glu-Glu-Phe-Tyr-Gly-Cys (Formula (VIII) in which R_4 is Glu-Glu, X is absent and Y is Gly-Cys; Seq. ID No. 28)

9a Pro-Gln-Gly-Pro-Gln-Ser-Met-Asn-Gly-Ala-Arg-Gly-Cys (Formula (IX) in which R_5 is Pro, R_6 is Arg, X is absent and Y is Gly-Cys; Seq. ID No. 29)

The peptides were cleaved from the solid resin support using 95% trifluoroacetic acid. The purity of the peptides was assessed using reverse phase high performance liquid chromatography. All peptides were

90% + pure except for peptide 7a which could only be obtained at a purity of 41% due to difficulties in the synthesis.

Conjugation of Peptides to Ovalbumin and BSA.

Peptides were conjugated to ovalbumin and BSA using m-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS).

Ovalbumin: 100 mg of ovalbumin (Sigma Grade V) (2.3 μmol) and 10 mg of MBS (32 μmol) were mixed in 10 ml of a 0.1 M solution of phosphate-buffered saline (PBS) (pH 7.2). The activated carrier solution was dialysed and divided into 10 x 1 ml aliquots. 8 mg of peptide (50-75 μmol) in 0.05 M PBS (pH 6) was added to each aliquot. The peptide:carrier ratio was estimated to be 15:1.

BSA: 100 mg of BSA (Sigma Grade V) (1.5 μmol) and 10 mg of MBS (32 μmol) were mixed in 10 ml of a 0.1 M solution of phosphate-buffered saline (PBS) (pH 7.2). The activated carrier solution was dialysed and divided into 10 x 0.25 ml aliquots. 2 mg of peptide (12-20 μmol) in 0.05 M PBS (pH 6) was added to each aliquot.

Treatment Groups.

225 Atlantic Salmon, each of approximately 35 g in weight at the beginning of the trial, were split into 15 treatment groups of 15 individuals. Throughout the trial the fish were maintained in flowing fresh water at 10-15 °C.

Group		Treatment
1	vaccine 1	Peptide 9a + ovalbumin with FCA
2	vaccine 2	Peptide 1a + ovalbumin with FCA
3	vaccine 3	Peptide 2a + ovalbumin with FCA
4	vaccine 4	Peptide 3a + ovalbumin with FCA
5	vaccine 5	Peptide 4a + ovalbumin with FCA
6	vaccine 6	Peptide 5a + ovalbumin with FCA
7	vaccine 7	Peptide 6a + ovalbumin with FCA
8	vaccine 8	Peptide 7a + ovalbumin with FCA
9	vaccine 9	Peptide 8a + ovalbumin with FCA
10	vaccine 10	Peptide 8b + ovalbumin with FCA
11	vaccine 11	Peptides 1a + 2a + 9a + Adjuprime
12	vaccine 12	Peptides 1a + 2a + 9a + FIA
13	control 1	FCA + ovalbumin
14	control 2	FCA
15	control 3	Untouched

Fish in Groups 1-14 were inoculated on day 1 of the study. Fish were anaesthetised by dipping in benzocaine (50 mg/L) prior to all inoculations and bleeds. All inoculations, including the controls, were administered by intraperitoneal injection. Blood samples, not less than 200 µl, were taken from the caudal vein.

Fish in Groups 1-10 received 100 µl of peptide-ovalbumin conjugate with FCA (50 µl conjugate: 50 µl FCA). Each fish received the equivalent of approximately 35 µg of peptide.

Fish in Groups 11 and 12 received 130 µl of peptide-ovalbumin conjugates with Adjuprime and FIA respectively (65 µl conjugates: 65 µl Adjuprime or FIA). Each fish received the equivalent of approximately 35 µg of each peptides 1a, 2a and 9a.

Fish in Group 13 received 100 μl of ovalbumin with FCA (50 μl ovalbumin in PBS: 50 μl FCA). Each fish received the equivalent of approximately 75 μg of ovalbumin.

Fish in Group 14 received 50 μl FCA with 50 μl PBS.

ELISA Protocol.

Sera were assayed for anti-peptide antibody levels at weeks 8 and 20 post-immunisation by ELISA. In sera from Groups 1-10, peptides 9a-BSA and 1a-BSA through to 8b-BSA, respectively, were used as the coating antigen (concentration = 5 μg peptide/ml). In sera from Groups 11 and 12, peptide 9a-BSA was used as the coating antigen (concentration = 5 μg peptide/ml). In sera from Groups 13 and 14, ovalbumin was the coating antigen (concentration = 20 μg peptide/ml). In sera from Group 15, peptide 9a-BSA was the coating antigen (concentration = 5 μg peptide/ml). Anti-peptide antibodies were detected using a monoclonal mouse anti-salmon immunoglobulin-horseradish peroxidase conjugate (anti Ig/HRP) at a dilution of 1:4000, as described previously by Whyte et al. (1987). J. Fish Biol. **31A**, 185-190.

Neutralisation Assay.

An IPNV neutralisation protocol was established as follows. Chinook salmon (CHS) cells were obtained and a culture system established. IPNV was acquired from several sources and used to inoculate CHS cells to generate virus stock for the assay. IPNV supernatants were titrated using a microtitration technique in which monolayers of CHS cells grown on microplates were inoculated with various dilutions of virus. Cytopathic

effects (CPE) were assessed at each viral dilution and virus concentrations for the neutralisation assay calculated.

A microneutralisation assay was set up in which a constant amount of virus was incubated with an equal volume of diluted serum from a range of serum dilutions. After 1 hour the virus/serum combination was added to incubation medium on CHS cell monolayers in micro-cell culture plates. When CPE were observed in virus/cell control wells the monolayers were fixed in 10 % buffered-formalin and stained with a 1 % crystal violet soluton. Viral activity was measured by assessing cell lysis which was appraised by reading the absorbance of diluted dye from each layer at 600 nm.

The neutralisation titre was assessed from the serum dilution giving 50 % protection of cells from viral effects together with readings for monolayer absorbance above and below this titre. To express the titre in IPNV protection units/ml, the readings were multiplied by the inverse of the dilution and divided by the assay (μl) volume.

Results.

Antibody Titres.

Figures (1) and (2) show the antibody titres from sera taken at 8 and 20 weeks post-immunisation respectively. The sera from 10 fish in each group were assayed and the mean serum antibody titre ($-\log_2$) calculated.

At 8 weeks post-immunisation, there appeared to be little antibody production over and above that seen in the control groups. However, at 20 weeks post-

immunisation, good antibody production was elicited to IPNV peptides in Groups 1, 2, 4, 5, 7, 8, 9 and 10. Even in Groups 3 and 6, the antibody levels were almost twice those seen in the controls. These results indicate that a good anti-IPNV peptide antibody titre can be raised in salmon with a single inoculation of IPNV peptide conjugated to ovalbumin and administered with FCA.

Neutralisation.

Figures (3) and (4) show the neutralisation ability of sera taken at 8 weeks post-immunisation. The sera from 10 fish in each group were assayed and results expressed as mean serum protection units/ml.

The results in Figure (3) were obtained using an assay system in which the $TCID_{50}$ (i.e. the amount of virus that produces a cytopathic effect in 50 % of the cultured cells) was 9000. The results in Figure (4) used a $TCID_{50}$ of 1000.

The antibody titre at 8 weeks post-immunisation was not high and hence a good neutralising activity was not expected. However, there is nevertheless a significant degree of neutralisation of virus in sera from fish in Group 10, and from fish in Groups 2, 3, 8 and 10 (as can be seen from Figures (3) and (4) respectively), when compared with sera from the control Groups 13 and 15.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PROTEUS MOLECULAR DESIGN LIMITED
(B) STREET: Proteus House, Lyme Green Business Park
(C) CITY: Macclesfield
(D) STATE: Cheshire
(E) COUNTRY: England
(F) POSTAL CODE (ZIP): SK11 0JL

(ii) TITLE OF INVENTION: Synthetic Polypeptides

(iii) NUMBER OF SEQUENCES: 29

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
(EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9218163.5
(B) FILING DATE: 26-AUG-1992

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9219710.2
(B) FILING DATE: 17-SEP-1992

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Thr Asn Pro Gln Asp Lys Val Asn Asn Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Thr Asp Phe Ser Ser Asp Leu Pro Thr Ser Lys Ala Trp Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Thr Asp Phe Thr Ser Asp Leu Pro Thr Ser Lys Ala Trp Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Pro Thr Ser Lys Ala Trp Gly Trp Arg Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Thr Lys Tyr Gly Lys Tyr Asp Pro Glu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu Glu Val Ser Glu Ser Gly Ser Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln Glu Thr Ser Ser Tyr Thr Leu Glu Val Ser Glu Ser Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gln Glu Thr Ser Ser Tyr Asn Leu Glu Val Ser Glu Ser Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ser Arg Phe Thr Pro Ser Gly Asp Asp Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ser Arg Phe Thr Pro Ser Gly Asp Asn Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Pro His Gln Glu Pro Ala Pro Asp Asp Phe Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Pro His Gln Glu Pro Ala Pro Glu Glu Phe Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Pro Gln Gly Pro Gln Ser Met Asn Gly Ala Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Pro Gln Gly Pro Gln Ser Met Asn Gly Ala Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Pro Gln Gly Leu Gln Ser Met Asn Gly Ala Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Pro Gln Gly Leu Gln Ser Met Asn Gly Ala Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Gly Gly Gly Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Gly Pro Gly Pro Gly Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Gly Ser Ala Gly Ser Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Thr Thr Asn Pro Gln Asp Lys Val Asn Asn Gln Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Thr Asp Phe Ser Ser Asp Leu Pro Thr Ser Lys Ala Trp Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Pro Thr Ser Lys Ala Trp Gly Trp Arg Asp Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Thr Lys Tyr Gly Lys Tyr Asp Pro Glu Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Leu Glu Val Ser Glu Ser Gly Ser Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Gln Glu Thr Ser Ser Tyr Thr Leu Glu Val Ser Glu Ser Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Ser Arg Phe Thr Pro Ser Gly Asp Asp Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Pro His Gln Glu Pro Ala Pro Asp Asp Phe Tyr Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Pro His Gln Glu Pro Ala Pro Glu Glu Phe Tyr Gly Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Pro Gln Gly Pro Gln Ser Met Asn Gly Ala Arg Gly Cys
1 5 10

Claims

1. A synthetic polypeptide having at least one antigenic property of at least one strain of infectious pancreatic necrosis virus, said polypeptide consisting substantially of an amino acid sequence selected from sequences of Formulae (I) to (IX):-

Formula (I) (which contains Seq. ID No: 1)

X-Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Y;

Formula (II) (which contains Seq. ID Nos: 2 or 3)

X-Thr-Asp-Phe-R_1-Ser-Asp-Leu-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Y;

Formula (III) (which contains Seq. ID No: 4)

X-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Trp-Arg-Asp-Y;

Formula (IV) (which contains Seq. ID No: 5)

X-Thr-Lys-Tyr-Gly-Lys-Tyr-Asp-Pro-Glu-Gly-Y;

Formula (V) (which contains Seq. ID No: 6)

X-Leu-Glu-Val-Ser-Glu-Ser-Gly-Ser-Gly-Y;

Formula (VI) (which contains Seq. ID Nos: 7 or 8)

X-Gln-Glu-Thr-Ser-Ser-Tyr-R_2-Leu-Glu-Val-Ser-Glu-Ser-Gly-Y;

Formula (VII) (which contains Seq. ID Nos: 9 or 10)

X-Ser-Arg-Phe-Thr-Pro-Ser-Gly-Asp-R_3-Y; and

Formula (VIII) (which contains Seq. ID Nos: 11 or 12)

X-Pro-His-Gln-Glu-Pro-Ala-Pro-R_4-Phe-Tyr-Y

Formula (IX) (which contains Seq. ID Nos: 13, 14, 15 or 16)

X-Pro-Gln-Gly-R_5-Gln-Ser-Met-Asn-Gly-Ala-R_6-Y

wherein

R_1 is Ser or Thr;
R_2 is Thr or Asn;
R_3 is Asp-Gly or Asn-Ala;
R_4 is Asp-Asp or Glu-Glu-;
R_5 is Pro or Leu;
R_6 is Arg or Lys; and

X and Y may each independently be absent or independently be one or more amino acid residues, with the proviso that when present they do not form an epitope or part of an epitope of the VP2 or VP3 proteins of any strain of IPNV which, in the sequence of the VP2 or VP3 proteins of the strain, is contiguous with the sequence to which X and Y are attached; the polypeptide optionally containing a functionalised coupling moiety.

2. A polypeptide as claimed in claim 1 wherein in Formula (II), R_1 is Ser, in Formula (VI), R_2 is Thr and in Formula (VII), R_3 is Asp-Gly.

3. A polypeptide as claimed in claim 1 wherein in Formula (IX), R_6 is Arg when R_5 is Pro and R_6 is Lys when R_5 is Leu.

4. A polypeptide as claimed in any one of the preceding claims wherein X and Y are each independently absent or each independently represent less than 20 amino acids.

5. A polypeptide as claimed in claim 4 wherein X and Y are each independently absent or each independently represents between 1 and 6 amino acids.

6. A polypeptide as claimed in claim 5 wherein either X is absent and Y represents 1 or 2 amino acids or Y is absent and X represents 1 or 2 amino acids.

7. A polypeptide as claimed in claim 6 wherein in the sequence X or Y which represents 1 or 2 amino acids, the

amino acid or one of the amino acids provides a site suitable for attachment to a carrier.

8. A synthetic polypeptide having at least one antigenic property of at least one strain of infectious pancreatic necrosis virus, said polypeptide consisting substantially of an amino acid sequence selected from sequences of Formulae (I) to (IX):-

Formula (I) (which contains Seq. ID No: 1)

X-Thr-Thr-Asn-Pro-Gln-Asp-Lys-Val-Asn-Asn-Gln-Y;

Formula (II) (which contains Seq. ID Nos: 2 or 3)

X-Thr-Asp-Phe-R_1-Ser-Asp-Leu-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Y;

Formula (III) (which contains Seq. ID No: 4)

X-Pro-Thr-Ser-Lys-Ala-Trp-Gly-Trp-Arg-Asp-Y;

Formula (IV) (which contains Seq. ID No: 5)

X-Thr-Lys-Tyr-Gly-Lys-Tyr-Asp-Pro-Glu-Gly-Y;

Formula (V) (which contains Seq. ID No: 6)

X-Leu-Glu-Val-Ser-Glu-Ser-Gly-Ser-Gly-Y;

Formula (VI) (which contains Seq. ID Nos: 7 or 8)

X-Gln-Glu-Thr-Ser-Ser-Tyr-R_2-Leu-Glu-Val-Ser-Glu-Ser-Gly-Y;

Formula (VII) (which contains Seq. ID Nos: 9 or 10)

X-Ser-Arg-Phe-Thr-Pro-Ser-Gly-Asp-R_3-Y; and

Formula (VIII) (which contains Seq. ID Nos: 11 or 12)

X-Pro-His-Gln-Glu-Pro-Ala-Pro-R_4-Phe-Tyr-Y

Formula (IX) (which contains Seq. ID Nos: 13, 14, 15 or 16)

X-Pro-Gln-Gly-R_5-Gln-Ser-Met-Asn-Gly-Ala-R_6-Y

wherein R_1, R_2, R_3, R_4, R_5 and R_6 are as defined in claim 1 and X and Y may each independently be absent or each independently represents one or more amino acids with the proviso that, if X or Y are such that the region of homology with the sequence of the VP2 or VP3 protein of any strain of IPNV extends into the X or Y sequences, the region of homology must not be so long as to have a significant detrimental effect on the efficacy of the polypeptide as a vaccine and/or the ability of the polypeptide to elicit antibodies of high specificity.

9. A synthetic polypeptide as claimed in claim 1 or claim 8 of Formula (A):

$$[L_a\text{-}F]_m\text{-}[L_b\text{-}G]_n\text{-}L_c \qquad (A)$$

wherein F and G may each independently be a polypeptide according to any one of Formulae (I) to (IX) as defined in claim 1 or claim 8, L is a linking sequence, a, b and c are each independently 0 or 1 and m and n are each positive numbers.

10. A synthetic polypeptide as claimed in claim 1 or claim 8 of Formula (B):

$$L_d\text{-}[G\text{-}L]_m\text{-}F\text{-}[L\text{-}G]_n\text{-}L_e \qquad (B)$$

wherein F is a polypeptide according to any one of Formulae (I) to (IX) as defined in claim 1 or claim 8, G is a polypeptide according to any one of Formulae (I) to (IX) or other sequence, and m and n are each positive numbers.

11. A synthetic polypeptide which comprises an antigenically significant subfragment and/or antigenically significant variant of a polypeptide as

claimed in any one of claims 1 to 8.

12. A synthetic polypeptide as claimed in any one of the preceding claims additionally comprising at least one T-cell epitope.

13. An analogue of a synthetic polypeptide as claimed in any one of the preceding claims wherein X or Y include a retro-inverso amino acid.

14 A synthetic polypeptide or analogue as claimed in any one of the preceding claims coupled to a carrier.

15. An antibody or antigen binding fragment thereof which specifically binds to a synthetic polypeptide as claimed in any one of claims 1 to 14.

16. Anti-idiotypic antibodies which are specific to an antibody or antigen binding fragment thereof as claimed in claim 15.

17. A DNA molecule coding for at least one synthetic polypeptide as claimed in any one of claims 1 to 12.

18. A kit for detecting IPNV or antibodies against IPNV which comprises at least one synthetic polypeptide as claimed in any one of claims 1 to 14 and optionally comprises support means and/or a means of detecting binding of antibodies or antigen binding fragments to the synthetic polypeptides.

19. A kit for detecting IPNV or antibodies against IPNV which comprises at least one antibody or antigen binding fragment thereof as claimed in claim 15 and optionally comprises support means, at least one synthetic polypeptide as claimed in any one of claims 1 to 14, and\or means for detecting binding of antibodies or

antigen binding fragments to said synthetic polypeptide.

20. A pharmaceutical composition comprising as active ingredient at least one synthetic polypeptide as claimed in any one of claims 1 to 14 in association with one or more pharmaceutically acceptable adjuvants, carriers and\or excipients.

21. A pharmaceutical composition comprising as active ingredient at least one antibody or antigen binding fragment thereof as claimed in claim 15 in association with one or more pharmaceutically acceptable adjuvants, carriers and\or excipients.

22. A process for the manufacture of a polypeptide as claimed in any one of claims 1 to 14 which comprises coupling the residues using chemical, biological and\or recombinant techniques known per se and isolating the polypeptide.

23. A process for the manufacture of an antibody or antigen binding fragment thereof as claimed in claim 15 which comprises immunising a mammal with a synthetic polpeptide as claimed in any one of claims 1 to 14 and isolating the antibody formed or cells which produce the antibody.

24. A process for the preparation of an anti-idiotypic antibody as claimed in claim 16 which comprises immunising a mammal with an antibody or antigen binding fragment thereof as defined in claim 15 and isolating the anti-idiotypic antibody formed or cells which produce the anti-idiotypic antibody.

25. Use of a polypeptide as claimed in any one of claims 1 to 14 for the manufacture of a medicament for stimulating the piscine immune system for therapy or

prophylaxis of IPNV infection in fish.

26. Use of an antibody or antigen binding fragment thereof as claimed in claim 15 for the manufacture of a medicament for the therapy or prophylaxis of IPNV infection in fish.

27. A method of detecting IPNV or antibodies against IPNV or antigen binding fragments thereof, which comprises incubating a sample of tissue or body fluid of a fish with at least one polypeptide as claimed in any one of claims 1 to 14 and determining whether, and if desired the extent to which and/or rate at which cross-reaction between said sample and said polypeptide occurs.

28. A method of diagnosing IPNV infection in fish which comprises incubating a sample of tissue or body fluid of said fish with an effective amount of antibody or antigen binding fragment thereof as claimed in claim 15 and determining whether, and if desired the extent to which and/or rate at which, cross-reaction between said sample and said antibody occurs.

29. A method of stimulating the piscine immune system for the therapy or prophylaxis of IPNV infection which comprises administering an effective amount of a polypeptide as claimed in any one of claims 1 to 14.

30. A method for the therapy or prophylaxis of IPNV infection in fish which comprises administering to the fish an effective amount of an antibody or antigen binding fragment thereof as defined in claim 15.

FIG. 1

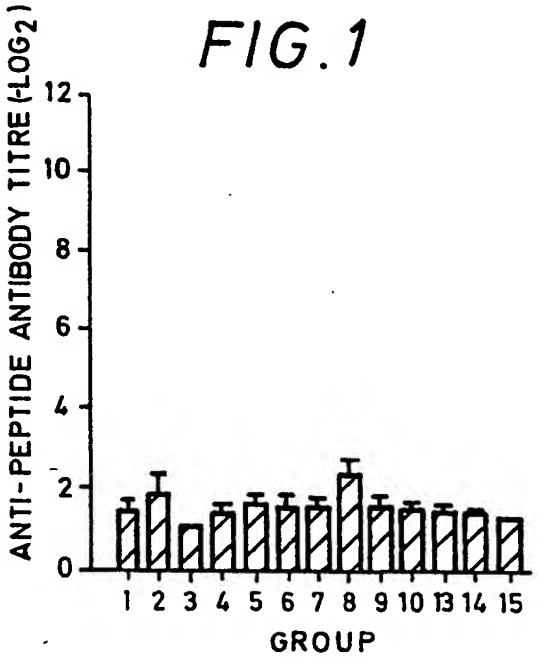

ANTI-PEPTIDE ANTIBODY TITRE (-LOG2)
0
2
4
6
8
10
12
1 2 3 4 5 6 7 8 9 10 13 14 15
GROUP

FIG. 2

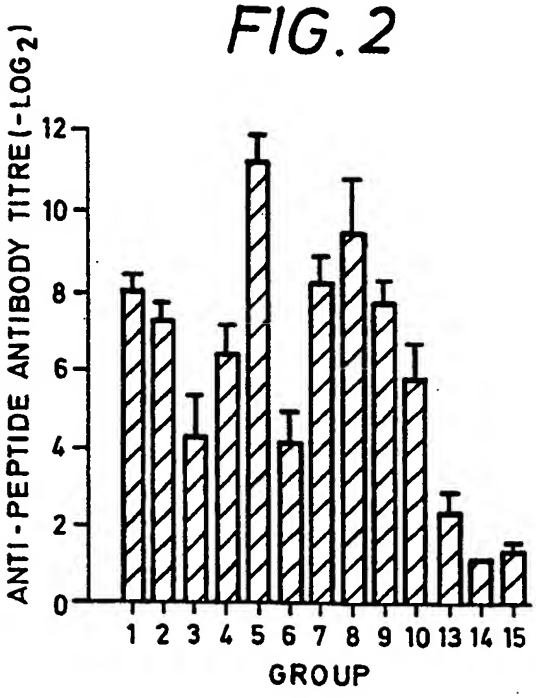

ANTI-PEPTIDE ANTIBODY TITRE (-LOG2)
0
2
4
6
8
10
12
1 2 3 4 5 6 7 8 9 10 13 14 15
GROUP

FIG. 3

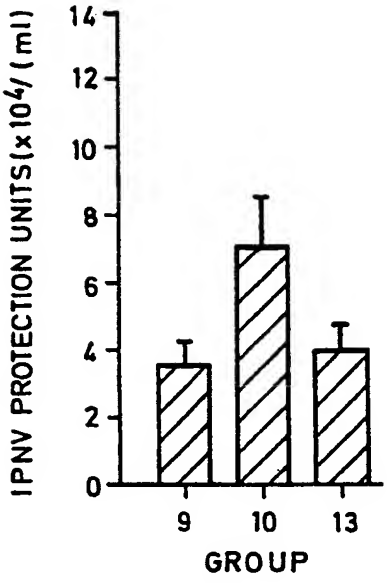
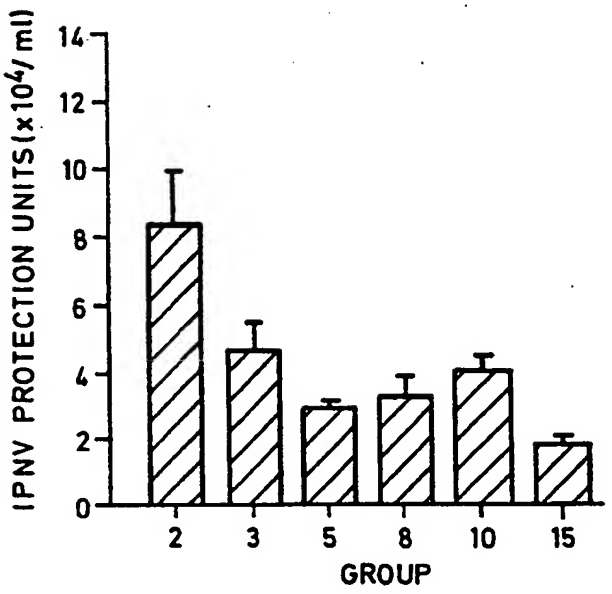

IPNV PROTECTION UNITS (x10⁴/ml)
14
12
10
8
6
4
2
0
9
10
13
GROUP

FIG. 4


IPNV PROTECTION UNITS (x10⁴/ml)
14
12
10
8
6
4
2
0
2
3
5
8
10
15
GROUP